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Title : EVALUATION OF ANTIOXIDANT POTENTIAL, IDENTIFICATION OF CONSTITUENTS AND STORAGE STABILITY OF ARTOCARPUS HETEROPHYLLUS J33 VARIETY FRUIT WASTE EXTRACT

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Artocarpus heterophyllus J33 (AhJ33) is an important fruit variety among A. heterophyllus in Malaysia and widely cultivated. For market purposes, the pulp is usually separated from the skin, which is usually discarded. Towards the conversion of AhJ33 fruit waste to a food product of high antioxidant potential, the specific objectives of this study were to evaluate the effect of three extraction methods on the antioxidant activity of AhJ33 fruit skin, isolate and identify the major and minor antioxidant constituents of the most active extract and evaluate its stability at three storage conditions. The fruit skin including rind and rachis were separated, dried, ground and extracted via maceration, percolation and Soxhlet techniques with 70% aqueous ethanol. Antioxidant activities were evaluated by DPPH, FRAP and b-carotene assays and correlated to their phenolic and polyphenolic contents which are estimated by TPC and TFC assays, respectively. The bioassay-guided isolation and identification of the major antioxidant constituents involved liquid-liquid partitioning (LLP) of the most active extract into ethyl acetate (EtOAc), dichloromethane (DCM) and aqueous (AQ) fractions followed by isolation and characterization of pure compounds via chromatographic and spectroscopic techniques, respectively. Minor antioxidant constituents of active extract were identified by TOF LCMS analyses based on total ion chromatograms (TIC) and LC-UV profiles at λ 254, 280 and 310 nm assisted mostly by built-in and online databases. The stability of stored extracts was evaluated by DPPH inhibition as well as qualitative and quantitative analyses of their major antioxidant constituents by LCMS and HPLC, respectively, throughout a period of 0-6 months. The results obtained showed that the maceration method produced extracts with the highest antioxidant activity which correlated well with phenolic, polyphenolic contents as well as LCMS profiles. The rind extracts showed higher antioxidant potential than the rachis extracts, with the rind maceration extract (RDM) showing the highest DPPH inhibition of 94.4% as well as highest values in FRAP and b-carotene assays. The strong correlation between antioxidant activities and phenolic and polyphenolic contents were indicated by high correlation coefficient values ($r>0.8$). The highest coefficient value was observed for DPPH-TPC with $r>0.99$. The order of antioxidant activity for the LLP fractions were EtOAc (94%) > DCM (88%) > AQ (43%) in the DPPH assay, and the same trend was observed in FRAP and b-carotene assays. Subsequent chromatographic separation of EtOAc fraction yielded a major compound characterized as protocatechuic acid (PCA). LCMS analysis of this fraction identified four more phenolic compounds namely chlorogenic acid, isovitexin, luteolin and feruloyl derivative along with 10 non-phenolics. The order of RDM stability at 6-month storage was frozen ≥ chilled > room illustrated by a 3.8-8.9% decrease in DPPH inhibition with a corresponding 2.5-6.3% drop in PCA concentration. This study suggests that AhJ33 variety rind extracts could be potentially developed as a food product due to its high antioxidant potential employing maceration as the extraction method. In addition, the extract should be stored at frozen or chilled conditions in order to retain the antioxidant activity above 90% over a 6-month period.